

## REMARKS

The last Official Action has been carefully considered. Reconsideration and allowance of the application in view of the amended claims and the following remarks is respectfully requested.

Claims 4, 6-8, 15, 21-24 and 34-37 are pending in the application. Claims 8, 34, 35, 36 and 37 have been amended. No claims have been cancelled.

It is noted that the last Office Action responds the applicant's amendment filed together with an RCE on November 30, 2009. The Office Action dated February 7, 2010 following the filing of applicant's RCE was withdrawn by the Examiner.

It is noted that claims 4, 6-8, 15, 21-24 and 34-37 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

It is further noted that claims 4, 6-8, 15, 21-24 and 34-37 are rejected under 35 U.S.C. § 103(a) as being unpatentable over US Patent No.: 6,130,101 to Mao ("Mao") of record.

Record is also made of an interview that took place with the Examiner on May 5, 2010 in which applicant argued against the Mao reference specifically also with respect to the impossibility of an ortho-position of the additional -COOH group in Mao. Applicant wishes to thank the Examiner for the courtesies extended to counsel at that time. As a result of the interview, the Examiner decided to withdraw the final Office Action of February 7, 2010.

As also submitted during the interview, the cited Mao reference points out the possible formation of lactones and lactam in col. 1 lines 63-65. Lactones and lactams are not interesting for analytical purposes since they show no absorption. Formation of lactone results from the ring formation of the ortho-carboxy group. Carboxy groups in meta- or para-position are not suitable for lactone formation due to the high tension in the ring.

In view of the above amendments to the claims, they now clearly distinguish from the teaching of the Mao reference as set forth in more detail herein.

**REJECTION OF CLAIMS 4, 6-8, 15, 21-24, AND 34-37 UNDER 35 U.S.C. §112,  
SECOND PARAGRAPH AND NON-COMPLIANT AMENDMENT**

Applicant submits that claims 34 and 35 as currently amended overcome the rejection on formal grounds. In amended claim 34, the former amended definitions for  $R_2$  and  $R_{12}$  are now properly underlined, although they were previously introduced; Cyc2 also includes the structure (G) as set forth in claim 34. Both (D) and (G) are now underlined to comply with the proper amendment practice.

In claim 35, an independent claim, the formula has been corrected to formula (Ia). Formula (Ia) was previously introduced by amendment dated May 13, 2009 but was inadvertently incorrectly set forth in applicant's last response as formula (I). Claim 35 specifies that the Cyc1 is substituted at the ortho-position of the ring attached to the backbone of formula (1a) with  $-\text{CONR}_5\text{R}_6$ . Support for this is found in paragraph [0004] through [0009] of the specification. In addition, this can also be seen from Examples 1-30 and 37-60.

Claims 36 and 37 were amended to correct minor punctuation problems. These amendments are cosmetic in nature.

Withdrawal of the rejection of claims 4, 6-8, 15, 21-24, and 34-37 under 35 U.S.C. §112, second paragraph and allowance thereof are respectfully requested.

**REJECTION OF CLAIMS 4, 6-8, 15, 21-24 AND 34-37 UNDER 35 U.S.C.  
§103(a) AS OBVIOUS OVER MAO**

Mao et al. disclose rhodamine dyes and dye conjugates for use in biological systems. Mao et al. explain the structure of rhodamines and refer to

the possible formation of the lactones and lactams (Mao et al. column 1, pages 63-65).

Today, rhodamine B is manufactured industrially by reacting N-(N,N-diethylamino)phenol and phthalic anhydride (Figure 1).

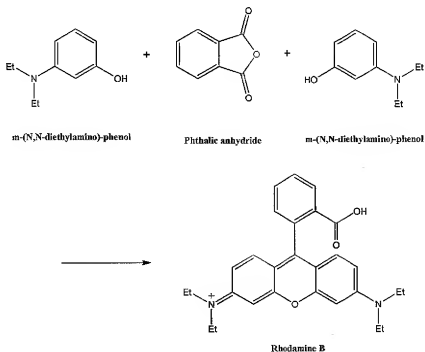


Figure 1

Because of this reaction procedure, rhodamine carries a carboxylic acid group in ortho-position to the xanthene system. By deprotonation of the carboxylic group in ortho-position, in an alkali environment the carboxy group may get deprotonized and absorption and fluorescence spectra may thus be shifted. In less polar environment, such as acetone, the carboxy group can react with the xanthene system thus forming a lactone. Lactones, however, cannot be used as dyes, since they do not fluoresce any more (Figure 2).

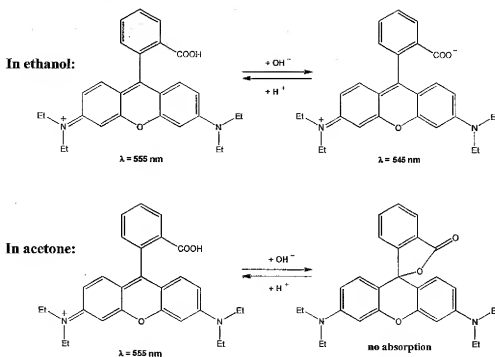


Figure 2: pH dependency of rhodamine B

To make biomolecules (proteins) microscopically visible, they are coupled to fluorescent dyes, preferably by an acid amide formation of the proteins amino group and a carboxylic acid substituted with dye. Such a coupling, however, does not function with the above rhodamine B, since the obtained acid amide at once is converted into a lactam, which does not fluoresce any more (Figure 3).

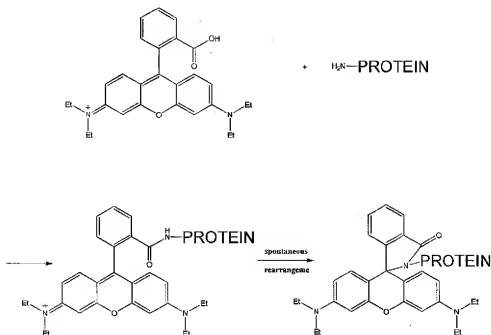


Figure 3: Coupling of rhodamine B to protein

In order to use rhodamine dyes which have excellent fluorescence properties, Mao introduces a further -COOH group into the rhodamine dye molecule. Such derivative is produced by using trimellitic acid anhydride instead of phthalic anhydride (Figure 4, Mao, Examples 1-3)

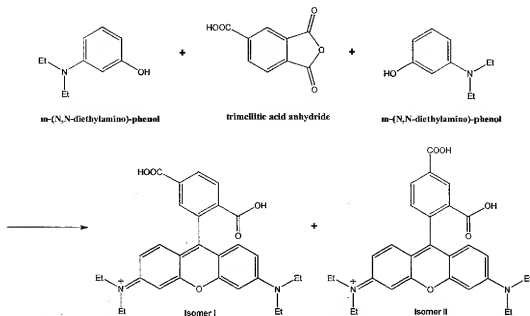


Figure 4: Synthesis of carboxy-rhodamine B

A mixture of two isomeric carboxy-rhodamines (Isomers I and II) is obtained which would have to be separated by chromatographic methods. Note that in any of the isomers, the additional -COOH group is either in meta- or in para-position to the xanthene group.

Contrary to the Examiner's position, which deems the "floating" -CONHX group in Mao *inter alia* in the ortho-position, the phenyl ring in Mao *et al.* is either 1,2,5-substituted (Isomer I) or 1,2,4-substituted (Isomer II). Based on the Mao synthesis of the compounds (using trimellitic acid anhydride), the substitution of the phenyl ring with the amide in ortho-position is not possible. Rather, the "floating" amide is either in meta (Isomer I) or in para (Isomer II) position. Moreover, attention is drawn to the fact that according to Mao *et al.* the dye further contains an ortho-carboxylic acid group, so that the above-described pH dependency (Figure 2) is not remedied.

In summary, Mao uses carboxy-rhodamines prepared using trimellitic acid anhydride. This production method is expensive and can only be performed in little scales. The Mao synthesis results in two isomers that must be separated by chromatographic methods. None of the isomers have two carboxylic acid groups in ortho position to the xanthene. Due to the ortho-substituted carboxy group, the Mao dyes are pH-dependent and may react to lactones that show no absorption at all (see Figure 2).

Generally, the rhodamine starting materials of the invention can be industrially produced and thus, are cost effective.

According to one aspect of the invention, the ortho-COOH group (that might be converted into a lactone) is modified in a simple manner by a secondary amine  $\text{HNR}_5\text{R}_6$  to a  $-\text{CONR}_5\text{R}_6$  group, wherein  $\text{R}_5$  and  $\text{R}_6$  independently is a hydrocarbon group (Figure 5).

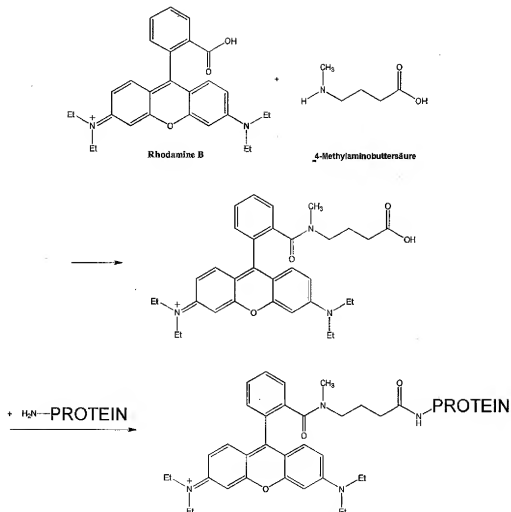


Figure 5: Coupling of rhodamine B according to the invention

Doing so the obtained products are not able to form lactams or lactones anymore due to the absence of a proton in the amide group. Accordingly, the advantageous optical features of the rhodamine can be maintained. Moreover, the carboxamide dyes of the invention do not have a pH dependency of absorption and fluorescence any more, since the ortho-carboxy group is not able to form rings.

By means of the presently claimed invention, all drawbacks of rhodamines having an ortho-COOH group are avoided. By converting the ortho-COOH group into a secondary amide, absorption and fluorescence become

independent of pH, since lactam formation is no longer possible, dye absorption and fluorescence may remain intact. They also remain independent of polarity. It is also possible to modify one of the residuals  $R_5$  and  $R_6$  with a functional group, for example, a carboxyl group. Such a group is incapable of forming a lactam or a lactone due to the large distance to the chromophore, thereby permitting coupling to a biomolecule or similar without any problems.

In brief, the Examples of Mao et al. do not comprise any compounds having a secondary carboxamide group in ortho-position. By this specific grouping, lactam- or lactone formation is prevented resulting in dyes having the above mentioned advantages. Moreover, the dyes according to the invention can be based on cost effective starting materials such as conventional rhodamine B.

## CONCLUSION

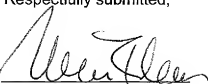
In view of the above, each of the presently pending claims in this application is considered patentably differentiated over the prior art of record and believed to be in condition for allowance. Reconsideration and allowance of the present application are thus respectfully requested.

Should the Examiner consider necessary or desirable any formal changes anywhere in the specification, claims and/or drawing, then it is respectfully requested that such changes be made by Examiner's Amendment, if the Examiner feels this would facilitate passage of the case to issuance. If the Examiner feels that it might be helpful in advancing this case by calling the undersigned, applicant would greatly appreciate such a telephone interview.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-1747.



Respectfully submitted,

By:   
Ursula B. Day  
Attorney for Applicant  
Reg. No. 47,296

Date: August 9, 2010  
708 Third Avenue, Suite 1501  
New York, N.Y. 10017  
(212) 244-5500  
UBD:pn